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Relationship of Chemical Structure to Corneal Penetration and Influence of Low-Viscosity Solution on Ocular Bioavailability

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Abstract □ Current understanding of the mechanism of corneal penetration by organic molecules proposes the epithelial layer as the rate-limiting membrane for water-soluble compounds and the stromal layer as rate limiting for lipid-soluble compounds. This suggests that the relationship between corneal permeability and the logarithm of oil/water partition coefficients, for a series of drugs, should not be the typical, single, continuous, parabolic-shaped curve. Corneal penetration studies have been conducted in unanesthetized albino rabbits using various organic compounds, representing five orders of magnitude in partition coefficient, at a constant concentration of 4×10^{-5} M dispensed in either a 1- or 90-centipoise (cps) solution. It has been shown that for non-ionizable compounds, a pair of bell-shaped curves were generated, one for lipid-soluble and one for water-soluble compounds. Small water-soluble species demonstrate very high apparent permeabilities, which may relate to the presence of aqueous pores or other paracellular drug movement. Penetration of ionizable compounds does not appear to correlate well with the structural relationships invoked for un-ionized compounds. Consistent with the proposed mechanisms of corneal penetration, oil-soluble drug substances show no improvement in drug bioavailability when dosed from a 90-cps solution, and water-soluble drugs show a modest improvement in ocular drug bioavailability. Small water-soluble substances demonstrate no improvement due to their already high bioavailability. The importance of nonproductive absorption and precorneal drainage on bioavailability is addressed.

Keyphrases □ Bioavailability, ocular—corneal penetration mechanisms, low-viscosity solutions □ Corneal penetration—ocular bioavailability, low-viscosity solutions

Although numerous studies on the penetration of organic compounds into the eye from topical dosing have been reported, these efforts have been limited in the majority of cases to the simple quantification of the amount of drug reaching the aqueous humor (1-3). None of these *in vivo* studies were designed to explore the relationship between drug structure and corneal penetration rate. On the other hand, there have been several reported *in vitro* studies which were expressly designed to explore corneal penetration rate and properties of the permeating species, especially the partition coefficient of the permeating species (4-6). In each of the *in vitro* studies, only a limited number of compounds and/or partition coefficient ranges was studied, and interpretation of the results generally ignored the mechanism of corneal penetration and precorneal disposition kinetics.

The intent of the present work is to explore corneal drug absorption as a function of drug properties, including the following specific aims: (*a*) an examination of vehicle effects (low-viscosity solutions) on drug bioavailability and the relationship of this effect, if any, to the mechanism(s) of corneal permeability, and (*b*) an investigation of the relationship of corneal penetration to physicochemical properties of the drug entity.

Several investigators (4-7) have postulated that the best predictor of corneal penetration rate of drugs is the oil/water (o/w) partition coefficient, and that an optimum partition coefficient exists for maximal penetration. Furthermore, several sources cite this optimal o/w partition coefficient for corneal penetration at a value of 10:1-1000:1 (4, 6), with the corresponding implication that very water-soluble drugs do not penetrate effectively. It has also been assumed that the charged forms of the drug, *i.e.*, ionized molecules, penetrate the cornea at a low rate that is essentially zero. In general, the studies upon which these assertions/assumptions have been based employed *in vitro* mounted corneas and either a restricted range of drugs (*i.e.*, a restricted range of partition coefficients) or a small number of drugs.

Based on published studies (8, 9) and the known structure of the cornea, a limiting-membrane mechanism for corneal penetration of small organic molecules, as depicted in Fig. 1, is proposed. When a drug with a high o/w partition coefficient is applied to the cornea, it quickly partitions into the "lipid-like" epithelial layers of the cornea. However, for such a drug, the aqueous stroma presents a rate-limiting membrane and, therefore, a small concentration gradient across this barrier is expected. Conversely, when a compound with a low o/w partition coefficient is applied to the corneal surface of high-

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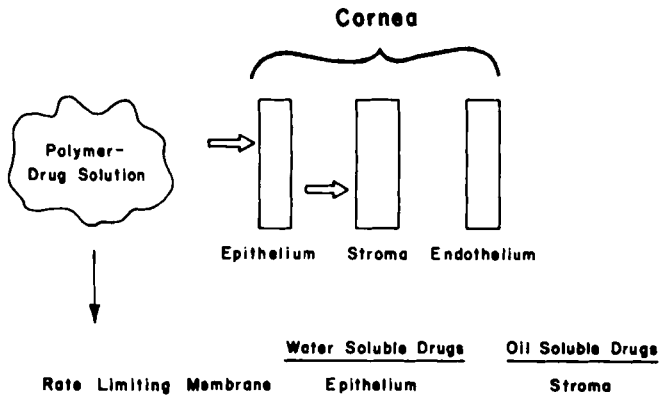


Figure 1—*Diagrammatic representation of proposed limiting-membrane mechanism for corneal penetration of small organic molecules based on oil/water partition coefficient for the penetrating species.*

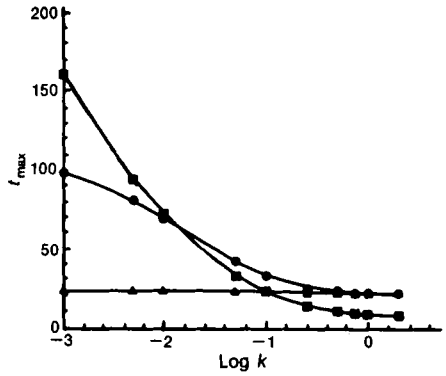


Figure 2—*Representation of the effects of changing rate constants on peak concentration (C_{max}) in the aqueous humor. Key: (●) k_{10}; (▲) k_{12}; (■) k_{23}.*

lipid character, this region serves as the rate-limiting membrane. Once across this barrier, the drug favorably partitions and quickly diffuses across the stroma. At some intermediate partition coefficient, it is likely that both membranes are rate limiting.

Directly related to this model are the anticipated effects of low-viscosity solutions on drug bioavailability. It is known that an increase in solution viscosity from 1 to 100 centipoises (cps) causes a decrease in precorneal drainage and, hence, increases corneal contact time for the drug. The decrease in the drainage rate constant, over this viscosity range, is ~10-fold in albino rabbits (10). For drugs with high o/w partition coefficients, little or no improvement in the bioavailability of the drug in the aqueous humor is expected, since the drug quickly partitions into the epithelium and the limited increase in contact time afforded by this solution should be of little or no benefit. However, water-soluble substances should show increased bioavailability due to the fact that any increase in contact time with the cornea allows continued flux of the drug across the rate-limiting membrane. Evidence of this result in rabbits has already been demonstrated for pilocarpine in low-viscosity polyvinyl alcohol and methylcellulose solutions (10–13).

A prediction of the effect of viscosity on bioavailability for compounds which possess both water and oil solubility (intermediate o/w partition coefficient) is difficult, since these drugs will most likely combine the above mechanisms. Predictions concerning ionizable compounds are equally difficult, since corneal penetration may involve mechanisms which are unrelated to the o/w partition coefficient. Expected corneal penetration mechanisms for the different drug types are summarized in Table I.

THEORETICAL SECTION

Pharmacokinetic Background—Many aspects of drug disposition in the eye have been reported (8, 15–17). Of particular interest is the precorneal fate and the rate of corneal absorption of various compounds. Extensive work in this area has been reported for pilocarpine, and this provides a substantial base from which to make predictions for other drugs (18).

One of the most important developments to come from the kinetic modeling of pilocarpine is the notion of a parallel loss process from the precorneal area. When a drug is placed in the precorneal area, in a simple solution, it experiences very short residence time. All corneal absorption of pilocarpine ceases after 4–5 min in rabbits (15). This, combined with the fact that most drugs demonstrate a relatively short time to reach peak levels in the aqueous humor, led early investigators to believe that the rate of corneal absorption was quite large (0.5 min^{-1}). However, this apparent rate constant and the true rate constant for corneal absorption have been demonstrated to be ~100 times smaller [~0.005 min^{-1} (15)]. The remaining rate of disappearance of drug from the precorneal area (0.495 min^{-1}) can be attributed to several loss processes, collectively referred to as the parallel loss function. This elimination step consists primarily of drainage coupled with tear turnover and nonproductive absorption. Since the rate constant for parallel elimination is ~100 times larger than the absorption rate constant, it is obvious that it is this larger rate constant which will control the time to reach peak drug levels in the cornea and the aqueous humor.

Since this is a parallel first-order process, the fraction of the dose absorbed can be calculated from:

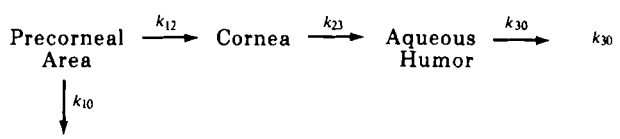
$$(F_t) = \frac{k_{12}}{k_{12} + k_{10}} \qquad \text{(Eq. 1)}$$

where k_{12} is the true rate constant for corneal absorption and k_{10} is the rate constant for precorneal loss. A simple calculation using the determined rate constants for pilocarpine predicts that the fraction absorbed should be ~0.01, and this has been found to be a good estimate (15). Assuming that k_{10} is largely comprised of the drainage rate constant (19), changes in the fraction of dose absorbed should be a direct reflection of changes in k_{12}.

An initial premise of the present work is that the true corneal absorption rate should be related to the o/w partition coefficient of the specific drug. Assuming this is accurate, it should be possible to predict changes in the extent of absorption from changes in the o/w partition coefficient.

Directly related to the above discussion is the expected effect of instilled solution viscosity on the extent of corneal absorption. Previous work with methylcellulose has demonstrated that a 100-cps solution can improve precorneal residence time by a factor of ~10, with a corresponding doubling of the aqueous humor levels of pilocarpine (10). Since an increase in residence time is associated with a decreased drainage loss, with subsequent smaller influence of tear turnover, this allows a calculated estimate of the maximum increase expected for this dosage form. Decreasing k_{10} by a factor of 10 and then calculating Eq. 1 gives a fraction absorbed of 0.09, or about a ninefold increase. It is emphasized that this calculation is for the maximum improvement obtainable and is dependent on the absolute magnitude of the corneal absorption rate. Just as the available time for corneal absorption increases so will that for nonproductive absorption, thus increasing drug loss by this mechanism. Also, drugs which could attain saturation of the epithelial layer would show varying degrees of improvement less than this amount. Since the degree of this saturation should be related to the o/w partition coefficient of the absorbing drug, very oil-soluble drugs should demonstrate little or no improvement, while very water-soluble compounds should approach this maximal increase. A gradation of this effect is expected for compounds with an intermediate o/w partition coefficient.

Theoretical Pharmacokinetic Simulations—As shown in Scheme I, a simplified model of the eye, based on previously published work with pilocarpine, was chosen to assess the importance of the various rate constants on drug levels in the aqueous humor. Drug movement is designated in one direction only, since there is no evidence of significant back-diffusion.



Scheme I—Model used to determine effects of parallel loss on C_{max} and t_{max} in aqueous humor

Table I—Expected Mechanism(s) of Corneal Penetration and Corresponding Viscosity Effects

Drug Type	Apparent Rate-Limiting Membrane	Mechanism	Low-Viscosity Polymer Influence
Water soluble	Epithelium	- Low oil/water partition into epithelium - High partition rate plus rapid diffusion through stroma-endothelium - Leaky channels do exist, *i.e.*, albumin (14) - Solute movement may be intercellular and/or transcellular	Increase in ocular bioavailability
Water and oil soluble	Epithelium–stroma	- Both mechanisms operate	Unknown
Oil soluble	Stroma	-High oil/water partition into epithelium and rapid diffusion through epithelium - Low oil/water partition into stroma	No effect
Ionizable	Epithelium or aqueous channel	- Mechanism not solely dependent on partition coefficient	Variable

The rate equation for appearance of drug in the aqueous humor is presented as:

$$dA/dt = k_{23}C - k_{30}A \qquad \text{(Eq. 2)}$$

where k_{23} is the rate constant for transfer from the cornea to the aqueous humor, k_{30} is the elimination rate constant from the aqueous humor, C is the amount of drug in the cornea, and A is the amount of drug in the aqueous humor at time t. C can be represented by:

$$C = \frac{Dk_{12}}{k_{12} + k_{10} - k_{23}} (e^{-k_{23}t} - e^{-(k_{12}+k_{10})t}) \qquad \text{(Eq. 3)}$$

as earlier derived (18), where D is the dose administered, k_{12} is the rate constant for corneal absorption, and k_{10} is the rate constant for precorneal loss. Equation 3 was integrated to give the amount of drug in the aqueous humor:

$$A = \frac{Dk_{12}k_{23}}{abc} (ae^{-k_{30}t} + be^{-k_{23}t} + ce^{-Kt}) \qquad \text{(Eq. 4)}$$

where a is $k_{23} - K$, b is $K - k_{30}$, C is $k_{30} - k_{23}$, and $K = k_{12} + k_{10}$. It should be noted that when D has a value of unity, A becomes the fraction of the applied dose in the aqueous humor at time t.

Simulations were conducted by solving for the fraction of dose in the aqueous humor at various times up to 60 min. Each rate constant was varied individually with the remaining values held constant, using the experimentally determined values for pilocarpine as starting values. A series of curves delineating the fraction of dose in aqueous humor *versus* time was generated for each rate constant examined. The elimination rate constant (k_{30}) was not varied.

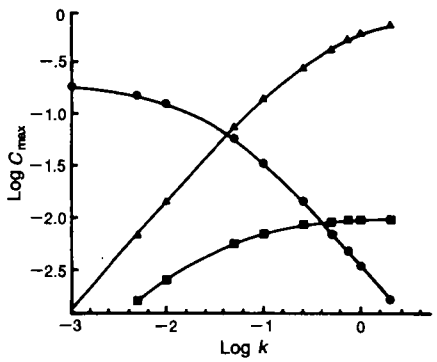


Figure 3—*Representation of the effects of changing rate constants on peak time (t_{max}) in the aqueous humor. Key: (●) k_{10}; (▲) k_{12}; (■) k_{23}.*

[1] Apple II+; Apple Computer, Cupertino, Calif.

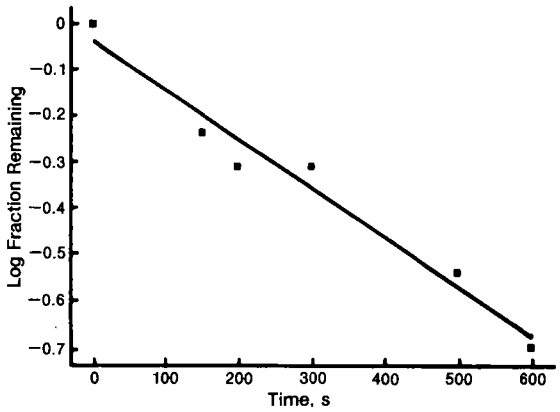


Figure 4—*Results of* in vitro *evaporation experiments for methanol conducted at 34°C. Note that the* SEM *is less than point size.*

In Figs. 2 and 3, the rate constant for parallel loss (k_{10}) is shown to affect both the time of peak concentration (t_{max}) and the magnitude of aqueous humor levels (C_{max}). The effect of decreasing the magnitude of this loss with, for example, a viscous solution or sustained-release system to a factor of 10 less, causes the expected increase in C_{max}. It is important to note that any decrease in k_{10} will be accompanied by a change in t_{max}. In this case, an order-of-magnitude decrease in k_{10} will result in an increase in peak time from the normal time of 20 min to ~40 min.

Results obtained for variations in the true absorption rate constant from its experimentally determined value of 0.005 min^{-1} demonstrate that the fraction of drug reaching the aqueous compartment is increased significantly with an increase in k_{12} (Fig. 3), as was expected from Eq. 1. Another result demonstrated in these simulations (Fig. 2), which would not be intuitively expected, is that t_{max} is minimally affected by increases in the range of values used for this rate constant. This is due to the fact that the changes in k_{12} are minimal when compared with the magnitude of k_{10}, which remains the controlling rate constant for precorneal processes. When k_{12} is increased by two orders of magnitude, this only doubles the sum of k_{10} and k_{12}; hence, the effects on t_{max} in this range of values are minimal. Therefore, it is expected that if changes in partitioning affect only the absorption rate constant, no effect on the time of peak levels in the aqueous humor should be observed. This is consistent with a previously presented theory (15).

The effects of a theoretical change in the rate constant for transfer of drug from the cornea to the aqueous humor (k_{23}) were as anticipated. At values below the experimentally determined value for pilocarpine (0.1 min^{-1}), t_{max} is increased and C_{max} is decreased. Conversely, t_{max} is decreased and C_{max} is increased for values >0.1 min^{-1}.

Summarizing the effects of changes in these rate constants on t_{max} and C_{max}, it may be concluded that an increase of the partition coefficient, which favors an increase in permeability through the corneal epithelium, will increase bioavailability of the compound (C_{max}), but should not influence the time to maximum aqueous humor concentration. This simulation does not allow for changes in nonproductive absorption due to changes in permeability from partitioning.

Table II—Characterization and Applied Concentration of Studied Compounds

Drug Name	Label	Supplier[a]	Specific Activity, Ci/mmol	Actual Applied Conc., $\times 10^{-5}$
Progesterone	Carbon-14	A	0.056	3.8
Fluorometholone	Tritium	A	23	4.01[b]
Fluorometholone acetate	Tritium	A	30	4.26
Hydrocortisone	Tritium	B	88	4.17
Hydrocortisone	Carbon-14	B	0.055	13.8
Prednisolone	Tritium	A	53	4.04
Methanol	Carbon-14	B	0.0036	46.80[b]
Ethanol	Carbon-14	B	0.021	7.64
Butanol	Carbon-14	B	0.0009	92.23[b]
Glycerol	Carbon-14	B	10	12.6[b]
Propranolol	Tritium	A	23	4.69
Pilocarpine	Tritium	B	6.95	4.00
Pyrilamine	Tritium	A	24.1	4.73

[a] A: New England Nuclear, Boston, Mass.; B: Amersham. [b] See text.

Table III—Thin-Layer Chromatography Results for Aqueous Humor Samples

Drug Name	Solvent System	Ratio	Metabolites Detected, %
Progesterone	Dichloromethane–acetone	4:1	<5.0
Progesterone	Ethyl acetate	—	<10.0
Fluorometholone acetate	Dichloromethane–acetone	4:1	<4.0
Fluorometholone acetate	Chloroform–ethyl acetate	3:1	<1.0
Hydrocortisone	Dichloromethane–acetone	1:1	<2.0
Hydrocortisone	Ethyl acetate	—	<1.0
Prednisolone	Ethyl acetate	—	<1.0
Glycerol	1-Butanol–water	9:1	<3.5

Factors which decrease the magnitude of drug loss will have a direct and almost linear effect on aqueous humor concentration and may increase the time to reach peak levels. A 10-fold decrease in k_{10} will result in a change in peak time from 20 min to ~40 min. Another factor of 10 decrease should result in a peak time of 90 min. It can be concluded, therefore, that a viscous solution that reduces the rate of loss from the precorneal area by a factor of 10, and increases C_{max}, should have a small effect on t_{max}. It is also notable that the second factor of 10 decrease in k_{10} (to a value of 0.005 min^{-1}) does not provide the same factor of nine increase in C_{max} decribed earlier, since it appears that for this rate constant an asymptotic value is reached (Fig. 3). This is reasonable and represents the ideal situation where no drug is lost, and k_{12} is the only precorneal rate constant for drug removal.

Due to the overwhelming magnitude of k_{10}, this rate constant has been proposed to control the peak time of drug in the aqueous humor. In cases where k_{10} is constant or decreases, this is valid. However, changes in k_{10} cannot induce a peak time in <20 min as demonstrated in Fig. 2. An increase in k_{23} is required to obtain a shorter peak time. The magnitude of k_{10} reaches an asymptotic value at 20 min. This value is obtained within the normal range of values expected for this rate constant, hence its control over C_{max}, t_{max} values below 20 min appear to be induced by an increase in the rate of transfer from the corneal epithelium to the following compartment. However, it is emphasized that the absolute magnitude of this asymptotic value of k_{10} is dependent on the fixed values of the remaining rate constants (k_{12} and k_{23}).

EXPERIMENTAL SECTION

Materials—Radiolabeled substances were obtained from commercial sources. All compounds were purified by vacuum distillation immediately prior to use. The original supplier, specific activity, and type of radiolabel are indicated in Table II for each compound. All other chemicals were either reagent or analytical grade and were used as received.

Male albino rabbits[2] (weight, 2.5–3.0 kg) were used throughout. Lighting and auditory background were maintained at a constant level in the caging facilities, and the animals were fed a regular diet with no restrictions on the amount of food or water consumed.

Preparation of Solutions—Drug solutions were prepared by the addition of purified, labeled material to a 4.0×10^{-5} M solution of the specific drug. The standard buffer solution was isotonic Sörenson's buffer at a pH of 7.4.

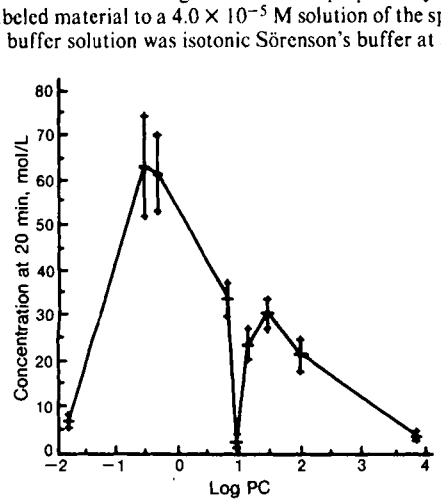


Figure 5– *Plot of molar concentration* versus *log partition coefficient for un-ionized species only.*

[2] Klubertanz, Edgerton, Wis.

Table IV—Effect of Solution Viscosity on Aqueous Humor Bioavailability

Compound	Log PC	C-20, × 10^9 1 cps	C-20, × 10^9 90 cps	Increase, %
Progesterone	3.89	3.25 (0.51)[a]	3.73 (0.38)	0[b]
Fluorometholone	2.00	23.72 (3.55)	29.84 (4.38)	0
Fluorometholone acetate	1.50	33.17 (10.57)	38.57 (6.83)	0
Propranolol	1.25	2.75 (0.34)	1.84 (0.25)	0
Hydrocortisone	1.20	25.80 (3.10)	33.46 (3.02)	0
Prednisolone	0.97	2.59 (0.57)	3.50 (0.48)	0
Pyrilamine	0.80	9.94 (1.44)	13.84 (1.53)	40[c]
Pilocarpine	0.22	5.56 (0.46)	10.84 (1.56)	95[d]
Methanol	−0.32	64.69 (10.90)	83.64 (18.90)	0
Glycerol	−1.79	6.84 (1.27)	8.15 (0.79)	0

[a] Numbers in parentheses represent *SEM*. [b] Percent increase was taken to be zero if no statistical difference was found between the means by t test. [c] $p < 0.01$. [d] $p < 0.001$.

Sufficient radioactive material was used to ensure good counting efficiency throughout the studies, *i.e.*, 100,000–400,000 cpm/μL of dosing solution. The actual final molar concentration of the solution was calculated from summation of the amount of unlabeled and labeled drug (Table II). Individual compounds, which resulted in high concentrations (Table II, footnote *b*) due to low specific activity, were also repeated at 20-min time points at another concentration to ensure linearity. All solution concentrations were normalized to 4×10^{-5} M for calculation and comparison purposes.

Ninety-centipoise solutions were prepared by the addition of methylcellulose[3] to a standard buffer solution until a final concentration of 0.8% polymer was reached. The viscosities of the solutions were determined with a viscometer[4].

Partition Coefficient Determinations—Oil/water partition coefficients were determined for all compounds listed in Table II. For lipid-soluble drugs, 4×10^{-5} M solutions of these compounds, with a sufficient amount of radiolabeled material added, were prepared in octanol. One hundred milliliters of this solution was added to an equivalent volume of isotonic Sörenson's buffer (pH 7.4) and allowed to reach equilibrium in a constant-temperature water bath at 34°C for 5–7 d. Both aqueous and octanol layers were sampled at 24-h intervals, and the samples placed in vials with scintillation cocktail for counting. The experimentally determined concentration was compared with the initial concentration in octanol, and an o/w partition coefficient was calculated. For water-soluble compounds, the procedure was the same except that the initial solution was prepared in the aqueous buffer phase.

Aqueous Humor Concentration Determinations—Rabbits were placed in restraining boxes to minimize movement. Doses (25 μL) were administered directly onto the corneas of both eyes with a micropipet. Animals were sacrificed at various times postdose by rapid injection of sodium pentobarbital solution through a marginal ear vein. The corneal surface was rinsed with buffer solution and carefully blotted dry with tissue. Aqueous humor samples were then tapped with a 1-mL syringe fitted with a 27-gauge needle.

Aqueous humor samples were transferred to glass vials, and scintillation cocktail[5] was added. Vials were dark-adapted for 24 h before counting to minimize chemiluminescence. Samples were counted with a liquid scintillation counter[6] and, after appropriate corrections, the final counts were converted to micrograms of drug per milliliter of aqueous humor.

Throughout the study, samples were removed as quickly as possible to minimize error due to redistribution and elimination. This is of particular importance for early time points and for drugs with apparently fast absorption kinetics. Rinsing the cornea prior to sampling and rapid surgery were methods used to minimize potential sources of error.

Metabolism Studies—Four aqueous humor samples were pooled, extracted with 1 mL of chloroform, and then centrifuged. The recovered chloroform was evaporated under nitrogen and then 10 μL of methanol was added. The methanol solution was spotted on silica gel plates[7] and developed in an appropriate solvent system. For water-soluble compounds (glycerol), aqueous humor was spotted directly without extraction. Control solutions were extracted, excepted glycerol, and treated in an identical manner. Dried plates were cut into 0.5-cm sections and then placed in scintillation vials containing 1 mL of methanol or water and mixed for 5 min. Scintillation cocktail was added and the samples counted after 24-h dark adaptation. Results were compared to TLC plates which had been spotted with nonradioactive solutions

[3] Methylcellulose E4-M Premium; Dow Chemical Co., Midland, Mich.
[4] Brookfield Engineering Labs., Stoughton, Mass.
[5] Aquasol; New England Nuclear, Boston, Mass.
[6] Packard Model 2002; Packard Instrument Co., Downers Grove, Ill.
[7] Polygram Sil G/UV_{254}; Brinkmann Instruments, Westbury, N.Y.

Table V—Molar Concentration of Drug in Aqueous Humor at Various Times Postdose

Compound	Log PC	Mol. Wt.	*C*-10, × 10^9 M	*C*-20, × 10^9 M	*C*-30, × 10^9 M	*C*-45, × 10^9 M
			Lipid Soluble			
Progesterone	3.89	314	1.62 (0.36)[a]	3.25 (0.51)	5.16 (1.31)	6.78 (1.55)
Fluorometholone	2.00	376	10.79 (1.62)	23.72 (3.56)	30.19 (4.45)	22.78 (3.71)
Fluorometholone acetate	1.50	418	—	33.17 (3.34)	—	—
Hydrocortisone	1.20	363	5.85 (0.89)	25.82 (3.10)	36.26 (7.41)	6.57 (1.34)
Prednisolone	0.97	360	3.04 (0.58)	2.59 (0.57)	3.84 (0.65)	2.89 (0.32)

			Water Soluble (Un-ionized) C-5 × 10^9 M	C-10 × 10^9 M	C-20 × 10^9 M	C-30 × 10^9 M
Butanol	0.88	74	—	56.43 (10.17)	35.47 (10.04)	—
Ethanol	−0.32	46	—	59.31 (10.57)	65.57 (8.71)	—
Methanol	−0.5	32	144.38 (12.55)	59.49 (13.97)	64.69 (10.88)	31.35 (11.45)
Glycerol	−1.79	92	—	3.12 (0.48)	6.84 (1.27)	6.32 (0.82)

				Water Soluble (Ionized) *C*-10,	*C*-20,	*C*-30,	*C*-45,
			pK_a	× 10^9 M	× 10^9 M	× 10^9 M	× 10^9 M
Propranolol	1.24	295	9.1	2.01 (0.50)	2.74 (0.34)	5.33 (0.76)	1.17 (0.24)
Pyrilamine	0.80	295	8.9	5.75 (0.99)	7.04 (1.01)	8.94 (1.86)	6.31 (1.01)
Pilocarpine	0.22	271	6.67	4.48 (0.37)	5.56 (0.46)	5.15 (0.73)	3.79 (0.54)

[a] Numbers in parentheses represent *SEM*.

and examined under UV light to determine R_f values for each drug in its specific solvent system.

***In Vitro* Evaporation Studies**—A 10.16-cm diameter petri dish was suspended in a water bath maintained at 34°C. Distilled water was added to just cover the inner surface of the dish. A 2.54-cm diameter watch glass was placed onto this arrangement and 25-μL samples of the test solution were placed on the watch glass and allowed to evaporate for ≤20 min. A separate watch glass was used for each determination to avoid contamination. At appropriate times, disks were rinsed with 3 mL of distilled water, and the rinsing solution was retained in a scintillation vial. Determinations of amount of compound remaining with respect to time were computed.

RESULTS

Drug Metabolism—Table III indicates, unexpectedly, that there is little or no metabolism occurring for the drugs examined over the time course of these studies. For example, published results (20) for prednisolone acetate demonstrate 100% metabolism under these conditions, and one would anticipate that fluorometholone acetate would behave similarly. Further comment on metabolism will be presented subsequently.

Viscosity Effects—Table IV gives the results of partition coefficient studies and aqueous humor levels for both 1- and 90-cps solutions. Results of partition coefficient studies correlate well with data available from Leo *et al.* (21). The percent bioavailability increase between 1- and 90-cps solutions was indicated as zero if there was no statistical difference ($p < 0.01$) in the mean values at 20 min as indicated by Student's *t* test. It is notable that a significant increase was demonstrated only for pilocarpine and pyrilamine. Additional studies for glycerol at both 20- and 30-min time points established that a change in t_{max}, expected from an increase in k_{10}, had not obscured the effects of viscosity on C_{max}.

Drug Concentrations in Aqueous Humor—Drug levels in the aqueous humor for the compounds studied are given in Table V. From the data, it appears that there is not a linear relationship between the partition coefficient and the amount of drug in the aqueous humor at 20 min. An order of magnitude increase in the partition coefficient does not coincide with a similar magnitude increase in the aqueous humor drug levels.

The low molecular weight alcohols (methanol, ethanol, and butanol) demonstrated peak times ≥10 min. Methanol and tritiated water data[8] from this laboratory had significantly ($p < 0.005$) higher levels in the aqueous humor at 5 min as compared with the 10-min levels. These compounds also have increased precorneal loss rates. *In vitro* determinations of evaporation rates for these compounds indicate that this is a significant mechanism of drug loss (methanol = 0.21 min^{-1}, water = 0.098 min^{-1}). An example of the evaporation study for methanol is given in Fig. 4. The rate constants of evaporation (k_{ev}) for these compounds were as expected, with the more volatile compound having the greater value. If these values represent an accurate approximation of evaporation rates *in vivo*, then the total rate constant for loss can be calculated as the sum of k_{10} and k_{ev}. For methanol, the total loss rate constant (0.71 min^{-1}) is ~40% higher than for nonvolatile compounds (0.5 min^{-1}).

To verify that methanol was not penetrating at a rapid rate due to toxic destruction of the corneal barrier, fluorometholone acetate was coadministered. Results were identical to fluorometholone acetate levels obtained without methanol.

The plots of C_{max} *versus* the log partition coefficient provide some insight into the relationships of the corneal penetration mechanism and the partition coefficient. Of particular interest is the observation that a clear dual-parabolic shape is demonstrated in the plot which includes only non-ionizable species (Fig. 5). This is consistent with predictions derived from the concept of the corneal epithelium or stroma as rate-limiting membranes depending on the partitioning properties of the absorbing drug. It is also important to note that on a molar basis, small molecular weight (water-soluble) species such as methanol and ethanol have greater corneal permeability than larger molecular weight compounds. This argues against the conclusions of some investigators, who have postulated that water-soluble compounds should show little or no corneal absorption. A similar relationship between partition coefficient and aqueous humor drug levels for ionized species is not clear, as shown in Fig. 6.

DISCUSSION

Vehicle Effects—Increasing vehicle viscosity from 1 to 90 cps did not result in an increase in aqueous humor levels for compounds whose log partition coefficients ranged from 1 to 4. This is consistent with the expectation that these lipid-soluble drugs quickly partition into the corneal epithelium. Thus, the limited increase in contact time afforded by low-viscosity solutions should not and does not improve their bioavailability. Propranolol data are also consistent with this expectation, since this compound has a relatively high partition coefficient. Despite the fact that propranolol is largely ionized at physiological pH, the favorable partition coefficient of the un-ionized species predicts that no effect is expected from an increase in viscosity of administered solution. It is anticipated that the un-ionized species will account for the majority of penetrating drug; therefore, the high degree of ionization also accounts for the decreased bioavailability of propranolol when compared with a steroid with a similar partition coefficient (such as hydrocortisone).

The effects of viscosity on water-soluble compounds indicate that, for these species, the mechanism(s) of corneal penetration are not well understood. Based on the partition coefficient alone, un-ionized water-soluble species such as glycerol or methanol are predicted to show modest increases in bioavail-

[8] Unpublished results.

ability from a viscous solution. Related processes, such as the possible water structuring of glycerol, may account for this discrepancy. Structured water could greatly inhibit transport of even a small molecule through a highly lipid membrane; also, water structuring may not be fully reflected in an equilibrium partition coefficient measurement.

For methanol, the apparently high rate of corneal penetration, in light of its small molecular size, suggests that this compound may be passing through aqueous channels. No significant improvement in bioavailability is demonstrated with a viscous solution. It is obvious that the rate of transfer from the epithelium to the stroma for this compound will be very high (high k_{23}). This explains the very short peak time and, to some extent, the substantial levels of compound in the aqueous humor. The lack of improvement with a low-viscosity solution for methanol suggests several possibilities. The first is that of a saturable mechanism within the epithelium, since a compound which already demonstrates saturable kinetics in the epithelial layer from a 1-cps solution should reflect little improvement from moderately increased precorneal residence time. This proposes that methanol enters the epithelium at a fairly fast rate to a maximum solubility of the compound. Partitioning across this barrier occurs, followed by very fast transfer from the epithelium to the stroma and aqueous humor. The relatively low partition coefficient and linear change in aqueous humor levels with changes in dosing concentration, however, argue against this explanation.

An alternate explanation for the lack of a viscosity effect on methanol bioavailability requires a calculated estimate for k_{12}. Using the peak concentration to give a rough estimate of the fraction of dose absorbed, since an accurate calculation of area under the curve is not possible, methanol demonstrates an ~30-fold increase over pilocarpine. Using Eq. 1, it is possible to calculate an estimate of k_{12}. For methanol, the total loss rate constant, including evaporation, is ~0.71 min^{-1}. This provides an estimate for k_{12} of 0.3 min^{-1}. Decreasing residence time by a factor of 10 and maintaining the evaporation rate constant reduces k_{10} to 0.26 min^{-1}. An estimate of the fraction absorbed from Eq. 1 predicts a value of 0.53 or a maximum increase of ~1.75. As with pilocarpine, this calculation represents the maximum increase expected with a 10-fold increase in precorneal residence time, neglecting all other precorneal factors. In light of the fact that pilocarpine demonstrates only a factor of two increase from a calculated maximum of nine, little increase should be expected with methanol. Close inspection of these calculations reveals that the increase in k_{10} is responsible for the dramatic decrease in the effect of viscosity. Since reduction of residence time should have no effect on the rate constant for evaporation (k_{ev}), the total loss rate constant remains relatively high. For example, if the approximation of the fraction of methanol absorbed has been overestimated by a factor of two (*i.e.*, k_{ev} decreases to 0.15 min^{-1}), this only changes the estimation of maximum benefit from a viscous solution to 2.1.

It is interesting that only pilocarpine and, to some extent, pyrilamine demonstrate vehicle effects in rabbits. Note that as the partition coefficient increases from that of pilocarpine to pyrilamine, the effect from a viscous solution decreases, as expected. Of importance also is the observation that as the percent increase falls below 50%, it becomes more difficult to discern statistical differences in the values between 1- and 90-cps solutions. This indicates that in an ideal situation, where errors in biological data were greatly reduced, the trends demonstrated for prednisolone and hydrocortisone could be the minimal increases in bioavailability expected for compounds with this range of partition coefficients. It should also be kept in mind that the rabbit, as a model for human ocular kinetics, apparently demonstrates much less sensitivity to viscous solutions than the human eye does for vehicles in this viscosity range (22).

Complicating the results for ionizable compounds is the possibility of penetration of ionized species through the cornea. Evidence of possible ionized drug movement through the cornea has been documented for cromolyn sodium[9]. This compound, with a pK_a of 2.0, is almost totally ionized at physiological pH; yet, significant drug levels in aqueous humor from solution dosing have been reported[9] in rabbits and confirmed in this laboratory.

In light of the results with compounds of relatively high log partition coefficients (1.0–4.0), one can visualize the epithelial layer acting in the same manner as a viscous solution for the ionizable water-soluble compounds, maintaining drug concentration at the rate-limiting membrane.

Partition Coefficient Relationships—The plot of aqueous humor drug concentration at 20 min *versus* partition coefficient for un-ionized compounds, Fig. 5, is a dual parabolic shape, consistent with expectations based on the proposed model of corneal penetration. In the high log partition coefficient range (1.0–4.0), a parabolic-shaped curve is indicated, although it is not as well defined as expected. It is obvious that compounds such as prednisolone and hydrocortisone do not penetrate the epithelial layer as well as fluorometholone or fluorometholone acetate, and that progesterone partitions well into the epithelial layer but has extremely poor partitioning characteristics from this layer to the stroma (decrease in k_{23}). The relatively low aqueous humor bioavailability for these drugs may be partially due to an increase in nonproductive absorption, since partitioning through these tissues, *e.g.*, the conjunctiva and nictitating membrane, will increase as partitioning increases into the epithelium.

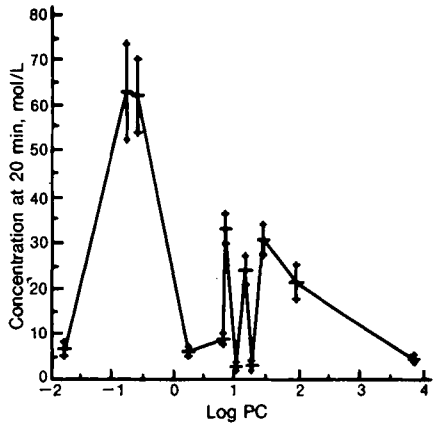


Figure 6—*Plot of molar concentration* versus *log partition coefficient for un-ionized and ionizable species.*

The curve depicted in Fig. 5 shows a much more defined peak than the *in vitro* data presented by Schoenwald and Ward (4). The reasons for this may be twofold. First, the plateau effect seen in the Schoenwald and Ward (4) data may be due to saturation of the system at partition coefficients near the "peak"; therefore, no defined peak is observed. Second, effects from changes in precorneal disposition cannot be accounted for in these *in vitro* studies. With a low partition coefficient, drug is lost to a greater extent by tear turnover and drainage. As the partition coefficient increases, corneal absorption increases in a relative manner, and relative drainage loss decreases. At very high partition coefficient values, nonproductive absorption greatly increases; hence, the sharp peak in the curve of the aqueous humor drug level *versus* the log partition coefficient.

As the o/w partition coefficient increases, the concept of a changing rate-limiting membrane predicts that the peak time should increase, since partitioning into the stroma becomes less favorable. This represents a decrease in k_{23} and should result in an increase in t_{max} and a corresponding decrease in C_{max}. It appears that this expected shift in peak time is obscured by the biological variability of the data. Although the means of the concentrations for the steroid compounds demonstrate a progressive increase in peak time with increasing partition coefficients, this is at a low level of significance ($p > 0.01$). In general, the expected decrease in C_{max} from a decrease in k_{23} appears offset by the increase in corneal absorption (k_{12}) due to favorable partitioning into the epithelium. The change in k_{12}, as previously noted, has no effect on peak time.

In the low log partition coefficient range (−2.0–1.0), a parabolic trend is also observed. This is most likely due to a combination of partitioning effects from the tears to the epithelium and then to the stroma. The peak in this region is due to the fact that compounds here have the best combination of partitioning characteristics in the water-soluble range. At the extreme ranges, drugs with the lowest partition coefficients cannot readily pass the epithelial layer (low k_{12}), and no matter how ideal their partitioning to the stroma (k_{23}), only low levels will be found in the aqueous humor. At the upper end of the water-soluble range, and the lower end of the oil-soluble range, it is probable that these compounds have both the epithelial layer and the stroma as rate-limiting membranes (low k_{12} and k_{23}), and therefore, have poor corneal permeability. The validity of the dual-parabolic shape of these results rests on the significance of the data for prednisolone and hydrocortisone. These data points are in substantial agreement with those of Schoenwald and Ward (4).

Water and Small Molecule Permeation—When low molecular weight alcohols were used, their rate of corneal penetration was much faster than the rate of most other molecules. Since the rate of elimination from the precorneal area by tear turnover should be essentially the same for these compounds as for all others, the shorter peak time observed must be due to an increase in transfer of drug from the epithelium to the stroma (k_{23}). Important to this discussion are results reported by Donn *et al.* (23) from a study utilizing $[^3H]H_2O$ and $[^{18}O]H_2O$. These investigators concluded that the transport for these two molecules was the same, within experimental error, thus ruling out the possibility of hydrogen ion transport as opposed to molecular transport

[9] V. H.-L. Lee, unpublished results.

for tritiated water. Significantly, they concluded that there is no active transport of water and that diffusion of water in the epithelium and endothelium is very close to the rate of diffusion for this molecule in the stroma, which is composed of 70–80% water. Since water and methanol are both compounds of small molecular size, the above comments concerning the possibility of paracellular penetration through the epithelium are strengthened. Such a pathway has been proposed by Kaiser and Maurice for fluorescein (24). This leads to a mechanistic picture of drug movement through the epithelium, where both transcellular and paracellular routes for drug permeation are possible and probable for water-soluble drugs. It is anticipated that transcellular drug penetration would be related to the partition coefficient, whereas paracellular movement would more likely be related to characteristics such as molecular size and aqueous diffusivity.

Ocular Metabolism—The results of metabolism studies in this work clearly indicate that, over the time period of 20–30 min in albino rabbits, the drug entities examined are not subject to appreciable degradation. Previously published work in the area of ocular metabolism must be considered before allowing such a simple conclusion. It is well documented that metabolism of pilocarpine is minimal in albino rabbits but increases in mixed breeds (25); since all work herein has been with albino species, this discussion will be restricted to studies using these animals. Studies in the area of ocular metabolism are limited in number, and the activity of different ocular enzymes appears variable; *i.e.*, pilocarpine undergoes minimal degradation, whereas dipivefrin demonstrates significant corneal metabolism (25, 26). The degradation of atropine is dependent on atropine esterase, which is inherited through an incompletely dominant trait (27). Mydriatic response to atropine is significantly less in a rabbit with positive esterase than one without the enzyme.

The area of steroid metabolism is equally confusing. One study (20) indicates that prednisolone acetate is metabolized completely to the parent alcohol, and one would therefore expect similar results with fluorometholone acetate. However, minimal metabolism was detected despite the fact that two different solvent systems were used which could potentially separate the acetate and alcohol forms. Another study (28) utilizing steroid alcohols and ketones, reports metabolites of $\leq 15\%$ from corneal and iris–ciliary tissue incubates. The results of these investigations agree with the present study for both hydrocortisone and progesterone. The subject of ocular metabolism, its mechanism and location, is one which requires additional research.

Dynamic *versus* Equilibrium Correlation—The original concept relating drug action to the partition coefficients, by Meyer (29) and Overton (30, 31), or to log partition coefficients, by Hansch and co-workers (32, 33), demonstrated relationships between lipophilic character and biological activity. Many of these examples deal with drugs that bind to enzymes, other proteins, or receptor sites. Current attempts to relate the partition coefficient and corneal penetration of compounds must rely on the fact that partitioning into the corneal layers mimics an equilibrium phenomenon: this is in fact difficult to imagine in the eye. Administered drugs have finite contact time with the corneal tissues. The dynamic aspect of corneal penetration is, therefore, being correlated with an equilibrium value of the partition coefficient; thus, one would not expect a good correlation between corneal absorption and the partition coefficient.

A significant rate of loss (k_{10}) controls the amount of drug reaching the target tissue, so steady-state kinetics, which should be accurately reflected by the partition coefficient, are not expected. Compounds with a very high rate of oil or water solubility can be expected to transfer more rapidly to their respective membrane of favorable partitioning. Therefore, some method of correlating the rate of partitioning with corneal penetration is desirable and is currently being pursued in this laboratory.

In summary, the present study has demonstrated that, as anticipated, a low-viscosity solution affords no benefit to compounds with a log partition coefficient >1.0. For these compounds, the rate-limiting membrane for corneal penetration is fairly well defined, although several factors influencing bioavailability, such as metabolism and nonproductive absorption, require further investigation and quantitation. Water-soluble compounds, on the other hand, are not as well understood.

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